CHEMOSYSTEMATICS OF PORIFERA: A REVIEW

R.W.M. VAN SOEST AND J.C. BRAEKMAN

Soest, R.W.M. van & Brackman, J.C. 1999 06 30: Chemosystematics of Porifera: a review. *Memoirs of the Queensland Museum* **44**: 569-589. Brisbane. ISSN 0079-8835.

All compounds isolated from Porifera were reviewed in an attempt to discover what level of reliability may be attached to chemistry data when applied to sponge systematics. To date (May 1998) more than 3500 different compounds have been described from 475 species of marine sponges, belonging to two of the three classes (Calcarea and Demospongiae), all major orders of Demospongiae, 55 families and 165 genera. Previous studies suggested that several ordinal, family and genus patterns may exist, with unique types of compounds apparently restricted to discrete sponge taxa. Based on this premise, the impressive chemical dataset is potentially valuable in solving persistent problems and disagreements over the systematics of various taxa. However, compounds may be produced by sponge cells (and thus regarded as sponge characters), or by microsymbionts (which may not be necessarily species- or group-specific). Large numbers of proven or suspected microsymbiont compounds appear to be present from the lack of correspondence between sponge identity and compound structure, e.g. macrolides and cyclic peptides dispersed amongst most demosponge groups are suspected products from various microbes. Reported chemistry is distributed heterogeneously over the various sponge taxa, with highest diversity of compounds reported from Dictyoceratida and Dendroceratida (1,250 compounds from 145 species), Haplosclerida s.l. (665 from 85 species) and Halichondrida s.l. (approximately 650 from 100 species); other groups have an intermediate (Astrophorida-Lithistida, Hadromerida and Poecilosclerida) or very low (Calcarea) diversity of compounds. Despite previous claims that particular compounds occur exclusively in particular sponge taxa, we found that in most, if not all, cases compound distribution does not exactly match sponge classification. Some classes of compounds are predominant in particular taxa (e.g. bromotyrosines in Verongida, furanoterpenes in Dictyo- and Dendroceratida, straight-chain acetylenes and 3-alkylpiperidine derivatives in Haplosclerida s.l.), but almost invariably there are also reports of these classes of compounds from unrelated sponges. Furthermore, in rare cases where a compound type is restricted to a certain sponge group (e.g. pyrrole-2-carboxylic derivatives in Halichondrida s.l.), their distribution amongst the families within the group appears to be inconsistent. Possible reasons for this fuzzy distribution include: 1) parallel biosynthetic pathways leading to the same structure; 2) involvement of microsymbionts; 3) careless specimen handling (contamination by epibionts, confused labels, etc.); 4) incorrect identification/classification. Currently, the degree of inconsistency is such that direct use of chemical data to solve classification problems, or to erect new higher taxa, is inadvisable. Inconsistent occurrence of compounds cannot be dismissed without further study. Large scale re-examination of voucher specimens, or recollection and chemical analysis, as well as cooperative studies between systematists, microbiologists and bio-organic chemists, are necessary to demonstrate whether or not chemical characters are true indicators of sponge systematics. ❐ *Porifera, chemistry, chemotaxonomy, bioactive compounds, review.*

Rob W.M. van Soest (email: soest@bio.uva.nl), Institute for Systematics and Ecology, University of Amsterdam, The Netherlands; Jean-Claude Braekman, Laboratoire de Chimie Bio-Organique, Université Libre de Bruxelles, Belgium; *4 April 1999.*

Natural products chemistry described from sponges is reminiscent of terrestrial plant chemistry in its diversity and distribution throughout the phylum. Secondary metabolites, such as terpenoids, alkaloids and peptides, as well as bioactive fatty acid-, polyketide- and sterol derivatives, are common amongst most sponge groups. Biological activity of sponge compounds is very diverse (MarinLit lists more than 20 activity categories for various sponge compounds; Blunt & Munro, 1998), but cytotoxic (see also Schmitz, 1994), antibiotic, antifungal, antitumour, antiviral, antifouling and enzyme- inhibitory activities are the most common.

Among marine organisms, sponges are the most productive sources of bioactive compounds: they

have so far yielded more than twice the number of structures reported from Cnidaria and from Algae, five times the number from Mollusca and Echinodermata, and seven times the number from Ascidiacea (Garson, 1994; Baker, 1996; Blunt & Munro, 1998).

Geographic areas and habitats with the highest reported numbers of bioactive compounds from sponges are the Indo-West Pacific (ca. 800 structures), Australian - South Pacific (600) and Caribbean coral reefs (600). The Mediterranean (550) and Japanese waters (750) are also prolific source areas. East Pacific (250), East Atlantic (150), Indian Ocean (150), Red Sea (150) and New Zealand waters (100) are intermediate in diversity. This pattern is similar to the pattern of sponge species diversity over the seas and oceans of the world (Van Soest, 1994), and thus cannot be directly linked to ecological phenomena such as increased predation and competition (e.g. Green, 1977).

Natural products continue to be described from sponges at an increasing rate (Table 1), such that the extent of sponge bioactivity is not yet apparent. So far, chemical structures have been elucidated from about 475 species of sponges, but many more have been shown to be bioactive in various bioassays.

Previous reviews (e.g. Bergquist, 1979; Bergquist & Wells, 1983; Sarma et al., 1993), demonstrated that many types of compound are restricted to discrete groups of sponges, the prime example being bromotyrosine derivatives which appeared to be restricted to Verongida. A large body of literature has appeared since these last reviews of sponge chemotaxonomy. This literature is now easily accessible through the MarinLit database (Blunt & Munro, 1998), which provides bibliographic references, structures and key words to virtually all marine natural products publications since the early sixties.

The origin of bioactive compounds isolated from sponges is still a controversial issue. Many bio-organic chemists believe that microsymbionts are likely to be the source of most compounds rather than sponge cells themselves. In some recent studies (e.g. Faulkner et al., 1994), it has been demonstrated that sponge microsymbionts may indeed be the source of bioactive compounds, but that sponge cells themselves also appear to produce them. It is possible, although yet to be demonstrated, that endosymbionts living inside the sponge cells are the true source of such compounds. In that case, such symbionts

TABLE 1. Numbers of published articles in which sponge chemistry is described and numbers of chemical structures reported in the past decades since 1960 (source MarinLit data base; Blunt & Munro, 1998).

Year of publication	No. articles	No. structures
1960-1969	4	2
1970-1979	314	361
1980-1989	1016	1275
1990-1997	1691	2484
total (1998)	3025	4122

may be obligatory symbionts which have co-evolved with the sponge hosts and sponge-symbiont chemical interactions may be indicative of chemo-taxonomic affinities. An example of such a scenario is explored in a recent study by Van Soest et al. (1998).

It is the purpose of the present paper to review conclusions of previous chemotaxonomic studies and to determine whether new chemotaxonomic evidence has come forward to support these conclusions. For this purpose we reviewed all sponge compounds and examined their distribution over the classes, orders, families and genera of sponges and, if relevant, over other marine phyla.

METHODS AND DATA SOURCES

The MarinLit database (Blunt & Munro, 1998) was consulted using taxonomic keywords for the various taxonomic groups yielding lists of references, species, and trivial names of compounds, as well as drawings of structures of compounds extracted from the species. The card system built up by one of us (JCB) was used as a supplementary source. These data provided a compilation of sponge chemistry arranged taxonomically by order, family and genus. Subsequent searches were made using trivial names of compounds or compound types as key words, to establish the distribution of classes of compound over the various sponge groups and other marine phyla. Since there is still no firmly established classification for sponges nor for secondary metabolite chemistry, chemosystematic significance of the various compounds and classes of compound was also determined by ad hoc discussions between the two authors.

Compounds considered to be related and occurring in two or more clearly different taxa (species, genera, families, orders) are listed

below arranged according to the sponge group in the order given in Table 2. The usually recognised sponge orders and families (Bergquist, 1978) are employed, with the exception of orders Halichondrida s.l. (following Van Soest et al., 1990), and Haplosclerida (following Van Soest, 1980). Orders Dictyoceratida and Dendroceratida are treated together for reasons explained below.

TABLE 2. Numbers of structures reported from various sponge taxa, arranged by ordinal group and the numbers of species, genera and families from which the compounds were isolated. * including Chondrosiidae; **including Halichondriidae, Axinellidae, Bubaridae, Agelasidae, Ceratoporellidae; *** including 'Nepheliospongida'/ Petrosida.

Taxon	No. Structures	No. Species	No. Genera	No. Families
Homosclerophorida	120	14	5	1
Astrophorida	200	38	14	4
Spirophorida	14	5	1	1
Lithistida	200	20	11	5
Hadromerida*	185	40	13	7
Halichondrida s.l**	650	100	23	7
Poecilosclerida	350	63	33	14
Haplosclerida s.l***	665	85	17	5
Lubomirskiidae	3	3	3	1
Dictyo/Dendroceratida	1,250	145	36	6
Verongida	240	22	8	3
Calcarea	40	9	3	2

Relatedness of compounds, in a phylogenetic sense, is not unequivocal as most compounds consist of building blocks and side-chains with often diverse biosynthetic origin. Unless biosynthetic experiments have been performed, homology of the seemingly related compounds remains tentative in most cases. In accordance with the chemical literature and to acknowledge discrepancies between chemical and morphological characters, we use the term 'markers' for shared compounds rather than 'synapomorphies'. Examples of structures of 'markers' for the various taxa are included (Figs 1-43). Unique compounds reported from single species, though possibly significant as fingerprints, are ignored here, because they cannot be used for classification.

RESULTS

NUMBERS OF COMPOUNDS ISOLATED FROM SPONGES. To date (May 1998) more than 3,500 different chemical compounds have been extracted from 475 species of marine sponges belonging to two of the three classes (Demospongiae and Calcarea), all major orders of Demospongiae, one major order of Calcarea, ca. 55 families and 165 genera (Blunt & Munro, 1998). The various orders demonstrate large differences in numbers of compounds (Table 2).

The total diversity of species compounds listed in Table 2 (3,917) maybe misleading because it has not been possible to check whether the same compounds were sometimes isolated from different sponge taxa. However, since that is of relatively rare occurrence, a conservative estimate is approximately 3,500 different structures. Similarly, the total number of sponge species from which these compounds have been isolated (544) is inaccurate because of the large numbers of indeterminate identifications; quite a few of these may concern the same species. A conservative estimate, based on arguments of geographic nearness of localities of indeterminate identifications, is approximately 475 different species of sponges.

GENERALLY DISTRIBUTED COMPOUNDS. The first category delineated includes compounds which are apparently found over several or many different sponge groups, without any clearly restricted distribution amongst any particular sponge group. Some of these are suspected to be products of microsymbionts because sponges are known to have a rich bacterial flora which they use as food. The chemosystematic significance of these types of compounds is usually limited to be, at most, a fingerprint for individual species. Frequently, however, even that cannot be confirmed because symbionts may not be species specific.

The following classes of compounds do not generally have much value for sponge classification because of their distribution amongst unrelated groups of sponges:

Fatty acids and derived lipids. (Fig. 1A). These are ubiquitous and are often primary metabolites, although some specialised branched or unsaturated fatty acids appear to be restricted in their distribution (see below). The chemosystematic significance of the presence and concentration of fatty acids with particular carbon-chain lengths has been explored by Bergquist et al. (1984), but from their patterns of distribution there is no hard

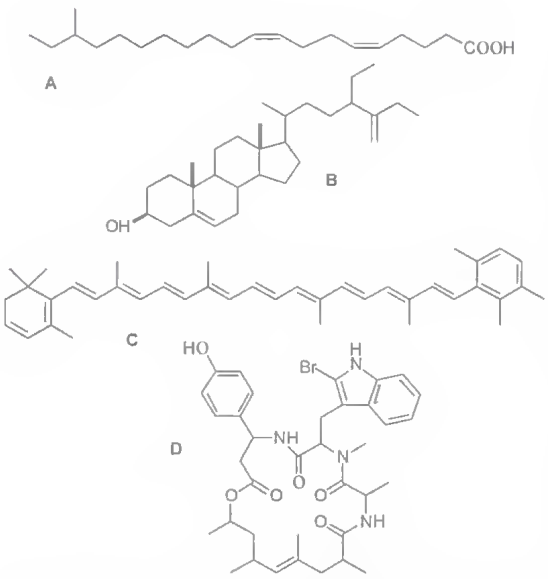

FIG. 1. Sponge chemistry. A, branched unsaturated fatty acid. B, sterol. C, carotenoid. D, cyclic peptide.

evidence for their applicability to sponge systematics.

Sterols. (Fig. 1B).These are ubiquitous and are often primary metabolites. Some sterols with specific side chains or functionalities (e.g. cyclopropene-, polyhydroxylated- or sulfated sterols) have a more restricted distribution and may have chemotaxonomic significance. Cyclopropene sterols have been used as a chemotaxonomic character to support the erection of a new order (Nepheliospongida or Petrosida; Bergquist, 1980), although subsequent research (Fromont et al., 1994) failed to demonstrate the consistent presence of these sterols amongst members of this 'order', whereas several similar cyclopropene sterols were isolated from the disparate taxa *Spheciospongia* (Hadromerida) (Catalan et al., 1982), *Halichondria* sp. (Halichondrida) (Ravi et al., 1978), and *Lissodendoryx topsenti* (Poecilosclerida) (Silva & Djerassi, 1991). The chemosystematic significance of the presence and concentration of sterols with particular side-chains and functionalities has been explored by Bergquist et al. (1986) and Fromont et al. (1994), but again there is no hard evidence for their consistency and applicability for sponge classification. They may be useful for fingerprinting at the species level, but even then care must be exercised (see e.g. Kerr & Kelly-Borges, 1994).

Carotenoids. (Fig. 1C). These are basically derived from ingested autotrophic organisms and modified in various ways by the sponges. A review is found in Liaaen-Jensen et al. (1982). The distribution of sponge carotenoids coincides with orange colour. They are reported from Astrophorida, Hadromerida, Halichondrida, Agelasida, Poecilosclerida, Haplosclerida, Dictyoceratida and Verongida.

Cyclic and linear peptides. (Fig. 1D). These elaborate molecules have been reported from Astrophorida, Spirophorida, Lithistida, Halichondrida, Poecilosclerida, Haplosclerida, Dictyoceratida and Dendroceratida. In a review published by Fusetani & Matsunaga (1993) it is concluded that microsymbiont involvement is the most likely explanation for the widespread occurrence of these compounds. Compounds similar to those isolated from sponges are also reported from Cyanobacteria and several other marine invertebrates, notably ascidians.

Macrolides. (Fig. 2). Similarly, these elaborate molecules have a wide distribution among Porifera: Calcarea, Astrophorida, Spirophorida, Lithistida, Hadromerida, Halichondrida, Poecilosclerida, and Dictyoceratida. Their apparent absence from Haplosclerida, Dendroceratida, Dysideidae and Verongida is noteworthy. Some of the molecules reported from sponges are almost identical to those of terrestrial Cyanobacteria or marine bacteria (Kobayashi & Kitagawa, 1998).

Acridine derivatives. (Fig. 3A). These compounds are not common, but recorded from Homosclerophorida, Astrophorida, Haplosclerida and ascidians. Some of these sponges and ascidians are brightly coloured due to the possession of acridine derivatives.

Nucleosides. (Fig. 3B). These have been recorded from Astrophorida, Hadromerida and Poecilosclerida. They are very likely to be microbial.

Sesquiterpene quinones. (Fig. 3C). Related structures have been recorded from *Chondrosia* (Hadromerida or Chondrosida), *Halichondria* (Halichondrida), *Strongylophora* (Haplosclerida) and many Dictyoceratida and Dendroceratida, and even from Verongida. No taxonomic significance or pattern can be attributed to this distribution.

Tetracyclic triterpenes. (Fig. 3D). Similar structures have been reported from *Siphonochalina siphonella* (Haplosclerida), *Axinella weltneri* (Halichondrida) and *Raspaciona aculeata* (Poecilosclerida). No taxonomic significance can be attributed to this distribution.

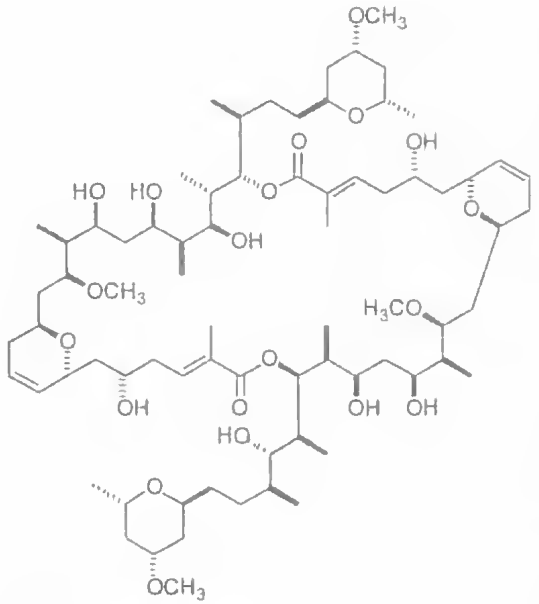

FIG. 2. Sponge macrolide.

TAXONOMICALLY DISTRIBUTED COMPOUNDS

Homosclerophorida compounds. About 120 different secondary metabolites have been reported from about 14 species belonging to 5 genera:

Peroxy-polyketides (recorded from at least 9 species belonging to at least 2 genera) and acridine derivatives (recorded from 4 species belonging to 2 genera), are common constituents of Homosclerophorida. However, both these types of compounds are also found in other sponge groups. The *Plakortis* peroxy-polyketides (e.g. Higgs & Faulkner, 1978) (Fig. 3E) are particularly similar to those isolated from *Callyspongia* sp. (Toth & Schmitz, 1994), *Cladocroce incurvata* (D'Auria et al., 1993) (both Haplosclerida) and from *Chondrosia* and *Chondrilla* (order Hadromerida or Chondrosida) (Wells, 1976; Stierle & Faulkner, 1979). So, despite the apparent concentrated occurrence of these structures in Homosclerophorida, it is not entirely justified to consider peroxy-polyketides as valid markers for the group. Further investigations are required as to why similar structures can be found in the unrelated Haplosclerida and Hadromerida/Chondrosida.

Aminosteroids isolated from *Plakina* sp. (Rosser & Faulkner, 1984: Fig. 3F) and *Corticium* sp. (Jurek et al., 1994), have an unusual nitrogen-bearing side chain, and these may be considered to be a valid marker for these two taxa despite their being sterols.

Astrophorida compounds. More than 200 secondary metabolites have been reported from at least 38 species belonging to 14 genera and all major families. Chemosystematic markers are listed below.

Saponines (steroid-saccharides, e.g. Carmely et al., 1989: Fig. 4A) are reported across families: from 6 species of *Erylus* (Family Geodiidae), 1 species of *Melophlus* (as *Asteropus*) (Family Ancorinidae) and 1 species of *Pachastrella* (Family Pachastrellidae). Related compounds are very common in Echinodermata, notably Asteroidea and Holothuroidea. Possibly the number of saccharides attached to the sterol part is species specific. The apparent absence of saponines in the other genera of the Astrophorida make them of dubious value for classification.

Triterpenes (malabaricane and derivatives, e.g. McCabe et al., 1982) (Fig. 4B) were reported from 2 species of *Stelletta*, 1 species of *Rhabdastrella* and from *Jaspis stellifera*. The latter is probably a *Stelletta* lacking triaenes and not a true *Jaspis*. Thus, these triterpenes are a good marker for *Stelletta* s.l. (including closely related

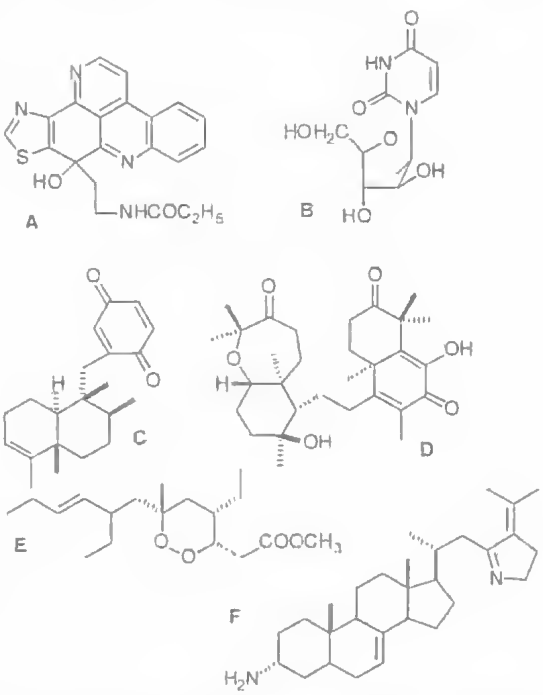

FIG. 3. Sponge chemistry. A, acridine alkaloid. B, nucleoside. C, sesquiterpene quinone. D, triterpene. E, peroxy-polyketide from *Plakortis halichondrioides*. F, aminosteroid from *Plakina* sp.

Rhabdastrella and '*Jaspis*' *stellifera*) (family Ancorinidae).

Penaresidins, peculiar straight-chained azetidine alkaloids (e.g. Kobayashi et al., 1991) (Fig. 4C) were independently isolated from two species of *Penares* (Ancorinidae) and thus may be a good marker for that genus.

There are also compounds suspected or proven to be of microsymbiont origin. Sulfated sterols were reported from *Pachastrella* and *Poecillastra*, and thus could be a potential marker for the family Pachastrellidae. However, sulfated sterols are also found in *Polymastia* (Hadromerida), *Hymedesmia* (Poecilosclerida) and several Halichondrida, so their value as marker is dubious. Also, like saponines, these compounds are very common in Echinodermata.

Cyclic peptides, macrolides and polyketides are commonly reported from species of *Geodia* (Geodiidae) and *Jaspis* (Coppatiidae), but these are often similar to Lithistid compounds and very probably of microsymbiont origin (discussed elsewhere in this review).

Geodia barretti and *Pachymatisma johnstonia* (Geodiidae) share a bromoindole compound of very similar structure, and thus these may be considered to be a valid marker for these two genera.

Dercitus (Pachastrellidae) and *Stelletta* (Ancorinidae) share similar acridine derivatives; however, related compounds are also reported from Homosclerophorida and Haplosclerida, as well as from ascidians as noted above.

Common-place sterols and (un)saturated fatty acids were reported from many species, but their chemosystematic value is low and they will not be discussed further here.

Spirophorida compounds. Fourteen secondary metabolites have been reported from about 5 species belonging to a single genus, *Cinachyrella* (Tetillidae). The fatty acids, sterols and macrolides shared between species do not seem to have chemosystematic value. The macrolides are similar to those of 'Lithistida' (e.g. *Theonella*), and to those isolated from the marine bacterium *Vibrio* sp. (Kobayashi & Kitagawa, 1998). No compounds are shared with the 'lithistid' family Scleritodermidae which is — on morphological grounds — assumed to be closely related to Spirophorida.

'Lithistida' compounds. Approximately 200 structures have been reported from at least 20 species belonging to 11 genera and 5 families. 'Lithistida' are certainly polyphyletic, with some

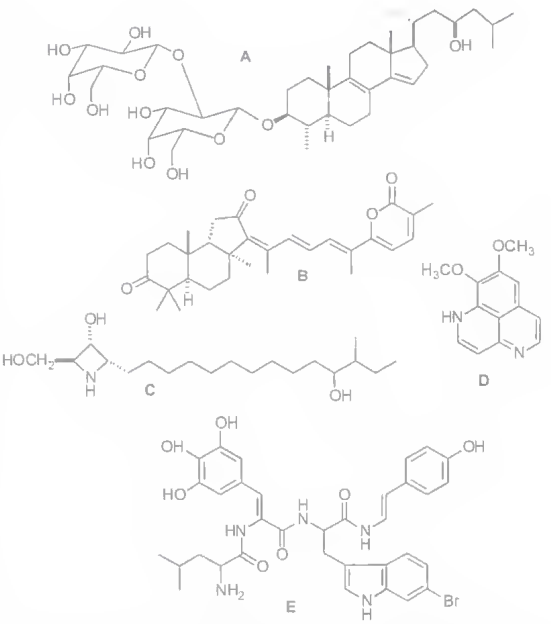

FIG. 4. A, Saponine from *Erylus lendenfeldi*. B, malabaricane-type triterpene from *Stelletta* sp. C, penaresidine from *Penares* sp. D, aaptamine from *Aaptos aaptos*. E, clionamide from *Cliona celata*.

families showing distinct synapomorphies with Spirophorida (e.g. Scleritodermidae) and Astrophorida (e.g. Corallistidae). Commonplace sterols were reported from several species, but their chemosystematic value is low and they will not be discussed further here.

Dominant compounds are cyclic peptides and macrolides, shared between families and genera. Related cyclic peptides are shared between several species of *Theonella*, 3 species of *Discodermia* and 1 species of *Neosiphonia* (Theonellidae), 1 species of *Callipelta* (Corallistidae), 1 species of *Aciculites* and 1 species of *Microscleroderma* (=*Amphibleptula*) (Scleritodermidae). Thus, it would seem that these cyclic peptides are straightforward markers for the 'Lithistida'. However, similar compounds are found in unrelated Astrophorida (*Geodia, Jaspis*), Hadromerida (*Hemiasterella*) and several Halichondrida (*Halichondria, Stylissa*). Discodermin E, the cyclic peptide isolated from *Discodermia kiiensis* (Ryu et al., 1994) is almost identical to the halicylindramides of *Halichondria cylindrata* (Li et al., 1995). These facts, coupled to the recorded presence of a rich microsymbiont flora in 'lithistids', support Fusetani & Matsunaga's (1993) conclusion of probable microsymbiont origin of these peptides.

Related macrolides are shared between several species of *Theonella*, 2 species of *Discodermia*, 1 species of *Neosiphonia*, 1 species of *Reidispongia* (Theonellidae) and 1 species of *Callipelta* (Corallistidae). Again, however, similar macrolides have been isolated from many different sponges belonging to widely divergent orders. As with cyclic peptides the chemosystematic value of 'lithistid' macrolides is thus compromised.

Hadromerida compounds. Approximately 185 secondary metabolites have been reported from at least 40 species belonging to 13 genera and 7 families. Fatty acids (except those mentioned below), sterols and carotenoids occur across several families and genera, but will not be discussed further. Cyclic peptides, macrolides and polyketides have been reported from a few Hadromerida and will also be left out of consideration.

No distinct hadromerid compounds can be identified. However, several compounds appear to be useful markers for families (or at least genus groups) and genera.

Aaptamine-type alkaloids (e.g. Nakamura et al., 198) (Fig. 4D) have been isolated from several species of *Aaptos* and *Suberites* and thus may be considered tentative markers for Suberitidae. They have been reported previously as markers for the order Hadromerida (Bergquist et al., 1991), but this is unwarranted in view of their limited occurrence.

Carballeira et al. (1989) maintained that 4,8,12-trimethyltridecanoic acid was a useful marker for the families Spirastrellidae and Clionidae, as they isolated this fatty acid from both *Anthosigmella varians* and *Cliona aprica*. The occurrence of this admittedly unusual fatty acid needs further investigation before this can be accepted.

Two presumably different species of *Cliona* apparently share the possession of clionamides (e.g. Stonard & Andersen, 1980) (Fig. 4E), which could serve as a useful marker for the genus. However, the amide shows some structural relationships with cyclic peptides and is thus a suspect microsymbiont-produced compound.

Peroxy-sesterterpenoids and derivatives (e.g. Capon et al., 1987) (Fig. 5A) have been isolated from 1 species of *Sigmosceptrella* and 3 species of *Latrunculia*. These genera were previously considered synonymous. But the value of these compounds as markers for Latrunculiidae is diminished by the isolation of closely related sesterterpenoids from 3 species of *Mycale* (Mycalidae) and from *Prianos* spec. (a name of uncertain affinity) (Manes et al., 1984).

Pyrroloquinoline alkaloids (e.g. Perry et al., 1986) (Fig. 18) have been isolated from 5 species of *Latrunculia*, but again the value of these compounds as markers for this genus is compromised by the reports of very similar and undoubtedly related compounds from another group of Poecilosclerida, viz. *Zyzzya* (Iophonidae) (see Van Soest et al., 1996; Dumdei et al., 1998).

If both compound types were genuine sponge compounds then their shared occurrence in Latrunculiidae - Mycalidae and Latrunculiidae - Iophonidae could indicate that 1) *Latrunculia* s.l. has poecilosclerid affinities; and 2) *Latrunculia* is polyphyletic supporting the data of Kelly-Borges. However, Perry et al. (1988) suggested that microsymbionts may be involved in the production of the pyrroloquinoline alkaloids.

Chondrosida compounds. This group is considered a family of the order Hadromerida in most previous classifications, but the apparent absence of morphological synapomorphies may justify their separation as an order of their own. Approximately 18 secondary metabolites have been reported from at least 4 species belonging to 2 genera.

Apart from straight-chained and branched unsaturated fatty acids and sterols, two other compound types have been reported from this group:

Peroxy-polyketides similar to, or some identical to, those of Homosclerophorida (e.g. Fig. 3E) were isolated from Australian *Chondrilla* (Wells, 1976) and Caribbean *Chondrosia* (Stierle & Faulkner, 1979). Mistaken identification is unlikely, though not impossible, and corroboration of the occurrence of peroxy-polyketides in Hadromerida/ Chondrosida would be welcome.

Halichondrida s.l. compounds. For morphological and chemosystematic reasons Halichondrida are here treated in a very wide sense, including the families Halichondriidae, Desmoxyidae, Dictyonellidae, Axinellidae, Bubaridae, Agelasidae and Ceratoporellidae. The nominal orders Axinellida (Hemiasterellidae and Raspailiidae excluded), Agelasida and Halichondrida s.s. are here included but not separately treated because the groups are in a taxonomic flux, with several recent revisions and proposed rearrangements. Morphologically, the recognised families are perceived by us to intergrade from Agelasidae at one end to Halichondriidae at the other end.

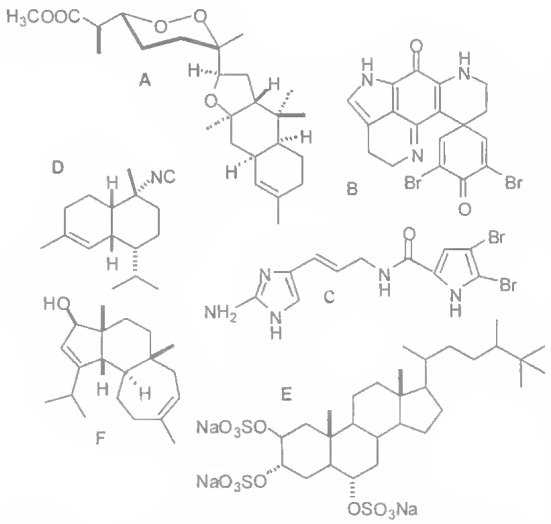

FIG. 5. A, trunculin-type sesterterpene from *Latrunculia brevis*. B, discorhabdin from *Latrunculia* sp. C, oroidin from *Agelas oroides*. D, isocyanosesquiterpene from *Halichondria* sp. E, sulfated steroid from *Halichondria* cf.*moorei*. F, cyclic terpene from *Myrmekioderma styx*.

Chemically there appear to be shared classes of compounds amongst family clusters with an overlap between Halichondriidae and Axinellidae (cf. Braekman et al. 1992, and see below). Many identifications of sponges with secondary metabolites are suspected or proven to be slightly or widely off the mark, which makes detailed re-examination of vouchers an essential prerequisite.

From Halichondrida, as employed here, approximately 650 structures have been reported from at least 100 species belonging to 23 genera and 7 families. Compounds with chemosystematic significance include the following.

Pyrrole-2-carboxylic derivatives (e.g. Braekman et al, 1992) (Fig. 5C) have been isolated from 12 species of *Agelas* (Agelasidae), 1 species of *Astrosclera*, 1 species of *Goreauiella* (Ceratoporellidae), 3 species of *Axinella*, 3 species of *Stylissa*, 1 species of *Phakellia*, 1 species of *Cymbastela*, 1 species of *Ptilocaulis* (as *Teichaxinella*) (Axinellidae) and 3 species of *Hymeniacidon* (Halichondriidae). At least one of the *Hymeniacidon* species (*H. aldis*) is a suspect *Hymeniacidon* as *H. aldis* is a junior synonym of *Stylissa massa*. It is possible that true *Hymeniacidon* (i.e., those with a detachable tangential skeleton) do not synthesise this class of compounds, and all reported *Hymeniacidon* with that compound type are in reality *Stylissa*. Thus, it appears that pyrrole-2-carboxylic derivatives are at least a marker for Agelasidae-Ceratoporellidae-Axinellidae, illustrated for example by the shared possession in *Agelas oroides* (Agelasidae), *Goreauiella* sp. (Ceratoporellidae) and *Stylissa carteri* (Axinellidae) of the same compound oroidin (Fig. 5C) (e.g. Braekman et al., 1992; Rinehart, 1989; Supriyono et al., 1995). A possibly related pyrrole compound is recorded from *Psendoceratina purpurea* (Verongida) (Tsukamoto et al., 1996), but it may also be a case of convergent synthetic pathways.

Isocyanoterpenes (Burreson et al., 1975) (Fig. 5D) have been isolated from 2 species of *Axinella*, 1 species of '*Stylotella*', 2 species of *Cymbastela*, 5 species of *Acanthella* (all Axinellidae), 1 species of *Bubaris* (Bubaridae), 5 species of *Halichondria*, 3 species of *Hymeniacidon*, 2 species of *Ciocalypta*, 1 species of *Topsentia*, 1 species of '*Leucophloeus*', 3 species of *Axinyssa* (partly as *Trachyopsis*), and 1 of *Epipolasis* (all Halichondriidae). Even though it is suspected that identifications may not be entirely accurate, this is overwhelming evidence, that isocyanoterpenes are shared between families Axinellidae and Halichondriidae.

Sulfated sterols (Fusetani et al., 1981) (Fig. 5E) were isolated from 2 species of *Halichondria*, 4 species of *Topsentia*, 1 species of *Axinyssa*, 1 species of *Epipolasis* and 1 Halichondriidae not further identfied. Thus it would seem that they are a marker for the family Halichondriidae. However, sulfated sterols are also common in Pachastrellidae (Astrophorida) and have been isolated from a species of *Polymastia* (Kong & Andersen, 1996) and a species of *Hymedesmia* (as *Stylopus*) (Prinsep et al., 1989); they are also common in Echinodermata.

Cyclic diterpenes (Sennett et al., 1992) (Fig. 5F) occur in *Myrmekioderma* and *Higginsia* and thus may be a potential marker for the family Desmoxyidae.

Linear diterpenes (Albrizio et al., 1992: Fig. 6A) described from *Myrmekioderma* and *Didiscus* appear to be unrelated or only distantly related to the cyclic diterpenes. Moreover, the record from *Didiscus* is a suspect identification because it concerns an E. Pacific species, and so far the genus *Didiscus* is not known from that area. It could be a case of a mistaken *Myrmekioderma*, because *Myrmekioderma* and *Didiscus* share similar habit characters. Consequently, the linear diterpenes may be a marker for *Myrmekioderma* only.

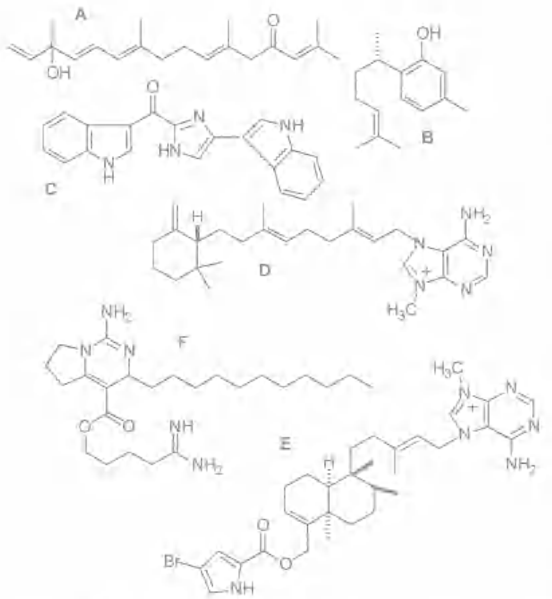

FIG. 6. A, linear diterpene from *Myrmekioderma styx*. B, curcuphenol from *Didiscus oxeata*. C, topsentin from *Spongosorites genitrix*. D, agelasine from *Agelas nakamurai*. E, agelasine G from *Agelas* sp. F, crambescine A from *Crambe crambe*.

Curcuphenol (Wright et al., 1987) (Fig. 6B) and related sesquiterpenes have been recorded from *Didiscus flavus* and *Epipolasis* sp. Some *Didiscus* specimens may have few of the characteristic didiscorhabs and then are easily mistaken for related genera such as *Topsentia* or *Epipolasis*. If that has been the case, it would mean curcuphenol and related sesquiterpenes are markers for *Didiscus*.

Topsentins (Bartik et al., 1987) (Fig. 6C) were considered a marker for *Spongosorites* since 4 species of that genus have yielded these compounds. However, this neat marker is threatened by the record of topsentins from 2 species of the Axinellidae genus *Dragmacidon*, which shows no close relationship with *Spongosorites*. The reported occurrence of both bromotyrosine derivatives (a Verongida marker compound) and topsentins in *Hexadella* (Dendroceratida) (Morris & Andersen, 1989; Morris & Andersen, 1990) is one of the more intriguing inconsistencies. It is also possible that bisindole compounds isolated from *Hamacantha* (Poecilosclerida) (Komoto & McConnell, 1988) are related to topsentins.

Terpene compounds (diterpenes (Wu et al., 1984) (Fig. 6D) and sesquiterpenes) are found in several *Agelas* species and thus may be markers of species groups within that genus (Braekman et al., 1992). A remarkable and significant compound was isolated from *Agelas* sp. (Ishida et al., 1992) (Fig. 6E): an apparent combination of a terpene and a pyrrole-2-carboxylic substructure. This indicates that in Agelasidae, in contrast to Axinellidae, some species have the ability to synthesise both terpenes and pyrrole-2-carboxylic acid moieties and even to combine these.

Compounds with low chemosystematic significance are the following: Macrolides and polyethers are commonly reported from *Halichondria*, cyclic peptides from *Halichondria*, *Axinella*, *Phakellia*, *Cymbastela* and *Stylissa*. Carotenoids are found in *Acanthella* and *Agelas*. Sterols and fatty acids are ubiquitous in this group. A host of unrelated smaller and larger compounds have so far been isolated from single species. Further exploration is needed to assess their potential as taxonomic markers.

Poecilosclerida compounds. Approximately 350 secondary metabolites have been reported from at least 63 species belonging to 33 genera and 14 families. Despite this large number of compounds, very few appear to have chemosystematic significance. Apart from sterols, fatty acids, macrolides and cyclic peptides, many indoles, pyrroles and carotenoids have been reported from Poecilosclerida, but in most cases there is no consistent taxonomic pattern.

Polycyclic guanidine alkaloids (Berlinck et al., 1990) (Fig. 6F) have been isolated from 2 species of *Monanchora* and 1 species of *Crambe* and are thus a potential marker for the family Crambeidae (Van Soest et al., 1996). However, these are also reported from *Arenochalina mirabilis* (Barrow et al., 1996), which is supposedly a Mycalidae. The voucher must be verified because *Arenochalina* has subtylostyles rather similar to those of *Monanchora* or *Crambe*, and reduced spiculation is very common in those genera.

Peroxy-sesterterpenoids and related derivatives (Capon & Macleod, 1987) (Fig. 7A) have been isolated from about 5 species of *Mycale*, but very similar compounds are known from *Latrunculia* (Hadromerida, see above and Fig. 5A). The chemosystematic value of these compounds is thus dubious.

Trikentrin (Capon et al., 1986) (Fig. 7B) and related compounds were isolated from two species of *Trikentrion* (Raspailiidae) and these might be a marker for that genus. But similar indoles are also reported for a species identified as *Axinella*

(Halichondrida) (Herb et al., 1990). Re-examination of the voucher might reveal that the characteristic triactines have been overlooked (they are often rare in various *Trikentrion* species and the further skeletal characters are similar to those of *Axinella*).

Haplosclerida compounds. Haplosclerida are here considered in a wide sense, including the order Nepheliospongida or Petrosida. The issue of one or two orders has been debated at several occasions using morphological, life cycle and chemistry arguments. Since both groups appear to share unique chemistry it is practical to unite the two groups for our purpose. From this group of 5 (marine) families, approximately 665 secondary metabolites have been reported from at least 85 species belonging to 17 genera and 5 families.

Chemosystematic markers appear as follows: straight-chain acetylenic compounds occur across 4 of the 5 families, they appear to be lacking in Niphatidae (see an extended review in Van Soest et al., 1998). The compounds are a clear marker for Haplosclerida s.l. However, related compounds have been described from *Phakellia carduus* (Halichondrida) and *Raspailia ramosa* (Poecilosclerida), which make it likely that the compounds are produced by microsymbionts. There are distinct types of acetylenic compounds based on the number of carbon atoms, the number and position of acetylenic bonds and the nature and position of the side chains. For example, *Petrosia* characteristically has hydroxyl-groups as side chains (e.g. Fusetani et al., 1983) (Fig. 7C), whereas the massive *Xestospongia* species (*X. muta*, *X. testudinaria*) characteristically have terminal bromine atoms (e.g. Patil et al., 1992) (Fig. 7D).

3-Alkylpiperidine derivatives have been reported from all 5 families of Haplosclerida and thus are a good marker for the order (see review in Andersen et al., 1996). Their occurrence in Phloeodictyidae is based on *Pellina* and *Pachypellina*, the family assignment of which is considered dubious. The type of *Pellina* is considered to be a *Halichondria*, but most species assigned to *Pellina* are either *Haliclona* (Chalinidae) or *Oceanapia* (Phloeodictyidae). The type of *Pachypellina* is considered to be a *Xestospongia* (Petrosiidae). It appears as if straight alkylpiperidines such as niphatesines (e.g. Kobayashi et al., 1992) (Fig. 7E) and halitoxins occur in families Niphatidae and Callyspongiidae, whereas cyclic alkylpiperidines (e.g. Sakai et al., 1986) (Fig. 7F) occur in Chalinidae, Petrosiidae

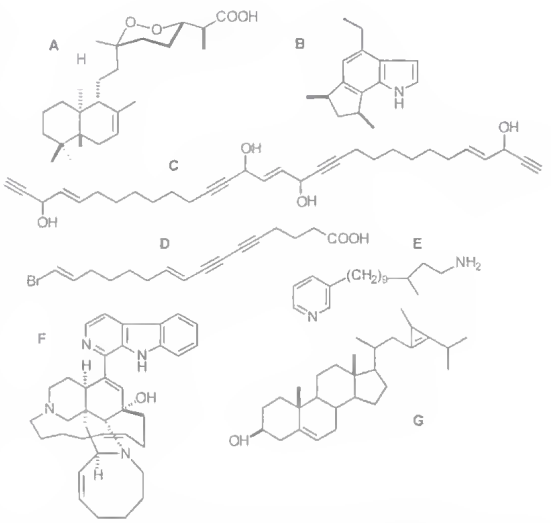

FIG. 7. A, sigmosceptrelline from *Mycale ancorina*. B, trikentrine from *Trikentrion flabelliforme*. C, straight-chain acetylene from *Petrosia* sp. D, straight-chain acetylene from *Xestospongia muta*. E, niphatesine D from *Niphates* sp. F, manzanine A from *Haliclona* sp. G, calysterol from *Calyx nicaensis*.

and (perhaps) in Phloeodictyidae. Much remains uncertain, because identification and assignment of species in this order are a specialist job. Voucher re- examination and repetition of collection and extraction is necessary to properly assess the chemotaxonomic significance of this group of compounds at the genus and family level. 3-alkylpiperidine derivatives have been reported from *Stelletta* (Astrophorida), *Theonella* ('Lithistida') and *Ircinia* (Dictyoceratida), but these are very likely cases of overgrowth by epibiont Haplosclerida, because identical compounds were also isolated from Haplosclerida. Unpublished information (M.K. Harper, in litteris) indicates that a particular 3-alkylpiperidine derivative, manzamine A, occurs also in a few Poecilosclerida (*Clathria* and *Mycale*). Thus, some doubts exist as to the true origin of these compounds, but no microsymbiont sources have so far been identified (see Kobayashi & Kitagawa, 1998).

A rather striking observation is that straight-chain acetylenes are recorded from several massive volcanoe-shaped *Xestospongia*, whereas 3-alkylpiperidines are recorded from compact, fine-grained and less elaborate *Xestospongia*. Previous authors have employed different names (*Xestospongia* s.l. and *Neopetrosia*) for these

sponges and chemistry appears to support this subdivision.

Cyclopropene sterols (e.g. Itoh et al., 1983) (Fig. 7G) are a marker for the 'order' Nepheliospongida/Petrosida (Bergquist, 1980), as they have been recorded from 2 species of *Xestospongia,* 2 species of *Petrosia*, 1 species of *Cribrochalina* (all Petrosiidae), 1 species of *Oceanapia* and from 2 species of *Calyx* (Phloeodictyidae). However, a recent study (Fromont et al., 1994) has shown that they are absent in most investigated members of these families. Moreover, similar sterols have been reported from species in the Hadromerida, Halichondrida and Poecilosclerida. Their chemosystematic significance is probably low and certainly debatable.

Tetrahydropyrans (e.g. Ciminiello et al., 1992) (Fig. 8A) are independently recorded from two species of *Haliclona.* They may be a marker for that genus, although such functionalities are widely distributed in natural compounds.

Low value markers for Haplosclerida are as follows: acridine alkaloids of closely related structure were isolated from *Amphimedon* sp. (Niphatidae) (Schmitz et al., 1983), *Petrosia* sp. (Petrosiidae) (Molinski et al., 1988), *Oceanapia sagittaria* (Salomon & Faulkner, 1996) and *Oceanapia* sp. (Eder et al., 1998) (Phloeodictyidae), and would seem to be a potential marker for those three genera/families. However, they contain an acridine moiety and are closely related to similar acridine compounds reported from Homosclerophorida and Astrophorida (as reported above).

Sesquiterpene and diterpene quinones are rather commonly found in Chalinidae (3 species), Petrosiidae (6 species) and Phloeodictyidae (2 species). However, similar compounds are also common in Dictyoceratida and Dendroceratida and are reported occasionally from Halichondrida and Chondrosida.

Isoquinolinoquinones are recorded from blue sponges assigned to *Reniera*, *Haliclona* (Chalinidae), *Petrosia*, *Xestospongia* and *Cribrochalina* (Petrosiidae). In view of the rather unusual blue colour, it is possible that these records all concern only a single species. In any case, identical or closely similar compounds are produced by a terrestrial *Streptomyces* (bacteria), and thus the chemistry is probably symbiont-derived.

The usual complement of fatty acids, sterols, cyclic peptides, polyketides and carotenoids have been reported across the families of Haplosclerida, but no taxonomic significance can be attributed to them. Many unrelated compounds were isolated from single species.

Freshwater sponge compounds. Only fatty acids and sterols have been reported from several species of the family Lubomirskiidae. These seem to have no taxonomic value.

Dictyoceratida and Dendroceratida compounds. We choose here to treat the orders Dictyoceratida and Dendroceratida in tandem because there is shared chemistry between the two and there is also a 'border conflict' over the assignment of the family Dysideidae. Bergquist (e.g. Bergquist, 1996) retains Dysideidae in Dictyoceratida, whereas Boury-Esnault et al. (1990) assign it to Dendroceratida. From this assemblage approximately 1,250 structures have been recorded from at least 145 species belonging to about 36 genera and 6 families.

Chemosystematic markers are as follows: Furan- or lactone terpenes (sesterterpenes: e.g. De Giulio et al., 1989 (Fig. 8B); sesquiterpenes, e.g. Guella et al., 1985 (Fig. 8C); and diterpenes: e.g. Bobzin & Faulkner, 1989 (Fig. 8D)) are shared by many species and genera of the group. Although there is a predominance of sesterterpenes in families Spongiidae, Irciniidae and Thorectidae (undisputed Dictyoceratida), a predominance of sesquiterpenes in Dysideidae, and a predominance of diterpenes in Darwinellidae and Dictyodendrillidae (both undisputed Dendroceratida), the occurrence is never absolute and many inconsistent records exist. Bergquist (e.g. Bergquist, 1996) chose to dismiss these inconsistencies announcing that they can be resolved by reassigning species to different families and genera. However, in view of the number of inconsistencies, this seems rather too optimistic. The evidence for this opinion is as follows. Sesterterpenic furans or lactones are found in: 11 species of *Spongia*, 3 species of *Hippospongia*, 3 species of *Carteriospongia*, 3 species of *Phyllospongia*, 1 species of *Strepsichordaia*, 1 species of *Collospongia*, 1 species of *Leiosella*, 1 species of *Dactylospongia*, 1 species of *Rhopaloeides*, 1 species of *Hyattella* (all Spongiidae), about 9 species of *Ircinia*, 3 species of *Sarcotragus*, 1 species of *Psammocinia* (all Irciniidae), 3 species of *Cacospongia*, 3 species of *Luffariella*, 3 species of *Fasciospongia*, 2 species of *Hyrtios*, 2 species of *Lendenfeldia*, 2 species of *Thorecta*, 1 species of *Petrosaspongia*, 1 species of *Fascaplysinopsis* (all Thorectidae), 2 species of *Dysidea*, 1 species of *Spongionella*

TABLE 3. Distribution of furan- and lactone terpenes over Dictyoceratida and Dendroceratida.

Compound	Spongiidae	Thorectidae	Ircinidae	Dysideidae	Darwinellidae	Dictyodendrillidae
Sesterterpene	26	17	13	3	0	2
Sesquiterpene	1	0	0	14	1	2
Diterpene	8	1	0	3	11	2

(Dysideidae), and 2 species of *Igernella* (Dictyodendrillidae).

Sesquiterpenic furans or lactones are found in: 1 species of *Spongia* (Spongiidae), 12 species of *Dysidea*, 2 species of *Euryspongia* (Dysideidae), 1 species of *Pleraplysilla* (Darwinellidae) and 2 species of *Dictyodendrilla* (Dictyodendrillidae).

Diterpenic furans or lactones are found in: 5 species of *Spongia*, 1 species of *Hippospongia*, 1 species of *Dactylospongia*, 1 species of *Hyattella* (all Spongiidae), 1 species of *Luffariella* (Thorectidae), 2 species of *Dysidea*, 1 species of *Spongionella* (Dysideidae), 3 species of *Aplysilla*, 3 species of *Chelonaplysilla*, 3 species of *Darwinella*, 2 species of *Dendrilla* (Darwiellidae), 1 species of *Igernella* and 1 species of *Dictyodendrilla* (Dictyodendrillidae).

From the overview presented in Table 3 it is evident that the number of cases that do not match the simple scheme presented by Bergquist (1996), viz. Dictyoceratida: sesterterpenes, Dendroceratida: diterpenes, Dysideidae: sesquiterpenes, is substantial, involving about 20% of all investigated species. It will take more than just reassigning a few possible mistakes. Moreover, the sesterterpenes, diterpenes and sesquiterpenes are biogenetically related. Several species (e.g. *Spongia agaricina*) apparently are able to synthesise both furanosesterterpenes and furanosesquiterpenes, or (e.g. *Spongia officinalis*) both furanosesterterpenes and lactone diterpenes. Because all three terpene types basically originate from a common biosynthetic pathway, which only at the end part of the synthesis of the terpenes will have divergent pathways, it is conceivable that the inconsistent occurrence of the terpenes is the product of independent (convergent) development. It seems best at present to emphasise the shared presence of furan- and lactone terpenes as a marker for both Dictyoceratida and Dendroceratida. A possible use of this compound type as marker for family or genus levels will have to await further studies combining voucher re-examination, morphological and molecular taxonomy, microsymbiont research and biosynthetic experiments. Pending this, it would be unwise to rearrange Dictyo- and Dendroceratida species and genera based only on terpene chemistry. Of course, emphasis of shared compounds which appear to confirm morphological synapomorphies remains a justified course of action.

A single inconsistent occurrence of sesterterpenic lactones is reported from a Japanese *Amphimedon* spec. (Ishibashi et al., 1993). This is possibly a case of mistaken labelling as the same group of chemists reported the occurrence of a typical Haplosclerid compound, manzamines, from an *Ircinia* spec. (Kondo et al., 1992).

Low value markers are as follows: Sesquiterpene quinones are reported from *Spongia* (4 species), *Hippospongia*, *Coscinoderma*, *Dactylospongia*, *Hyattella*, *Ircinia*, *Sarcotragus*, *Fasciospongia*, *Smenospongia*, *Hyrtios*, *Thorectandra*, *Fenestraspongia*, *Dysidea* (6 species) and *Euryspongia*. Thus, they seem to be good markers for Dictyoceratida including Dysideidae. However, these compounds are closely similar to the sesquiterpene quinones reported from Haplosclerida, Halichondrida and Chondrosida. Their apparent absence from Darwinellidae and Dictyodendrillidae is noteworthy.

Diketopiperazine derivatives, resulting from the condensation of two aminoacids, and diphenylether derivatives have been isolated from several species of *Dysidea*. However, sophisticated research by Faulkner et al. (1994) proved beyond doubt that bacteria are responsible for the production of these compounds. Diketopiperazines isolated from *Tedania* (although with different amino acid building blocks than those of *Dysidea*) also appeared to be produced by a bacterium (Stierle et al., 1991).

Polyhydroxylated sterols have been isolated from *Spongia* (2 species), *Hippospongia*, *Ircinia* (2 species), *Dysidea* (4 species), *Euryspongia* and *Spongionella*. Thus, they seem to be a marker for the Dictyoceratida including the family Dysideidae. However, these compounds are reported from isolated species belonging to almost all orders of the Demospongiae. Moreover they are commonly reported from Echinodermata and soft corals.

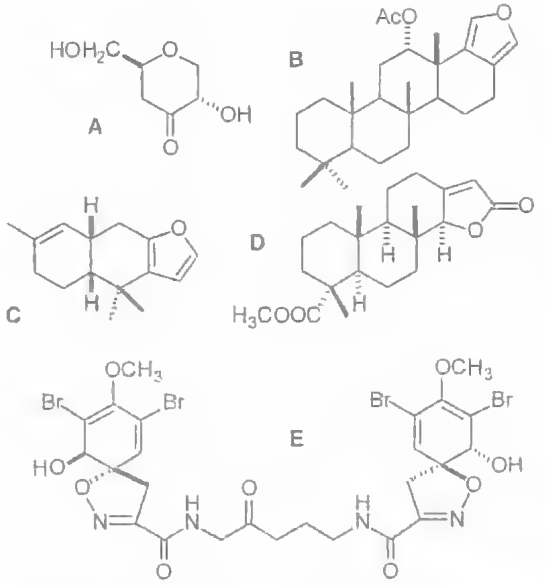

FIG. 8. A, halichondrol from *Haliclona hogarthi*. B, scalarine from *Spongia officinalis*. C, furanosesquiterpene derivative from *Dysidea avara*. D, polyrhaphin from *Aplysilla polyrhaphis*. E, bromotyrosine derivative from *Aplysina aerophoba*.

Indole derivatives occur scattered over all families. These compounds occur in many different sponge groups (and indeed other animal phyla), and they are assumed to be of microsymbiont origin. In any case, they appear quite diverse in the various species and genera.

Sterols and fatty acids have been isolated from many Dictyoceratida and Dendroceratida. Macrolides occur scattered over a few species in the families Spongiidae and Thorectidae; their absence in Dendroceratida is perhaps noteworthy. Cyclic peptides occur sparsely (here and there) over all families.

The family Halisarcidae is usually attributed to Dendroceratida, but was recently raised to ordinal level: Order Halisarcida Bergquist, 1996. No compounds have been isolated from members of this group so far.

Verongida compounds. Approximately 240 secondary metabolites are reported from at least 22 species belonging to 8 genera and 3 families.

Bromotyrosine derivatives (e.g. Ciminiello et al., 1997) (Fig. 8E) are uniformly present in all families and genera of Verongida (11 species of *Aplysina*, 2 species of *Verongula*, 2 species of *Ianthella*, 1 species of *Anomoianthella*, 2 species of *Pseudoceratina*, 2 species of *Suberea*, 1 species of *Aiolochroia* and 1 species of *Aplysinella*). Within this large group of derivatives, macrocyclic bromotyrosines (e.g. Pordesimo & Schmitz, 1990) (Fig. 9A) are shared between two species of *Ianthella*, so they could be a marker for that genus; however, a macrocyclic bromotyrosine is also recorded from *Pseudoceratina purpurea* (Carney et al., 1993).

The value of bromotyrosine derivatives as a marker for the Verongida is diminished by the isolated occurrence of similar bromotyrosine compounds in *Iotrochota birotulata* (Poecilosclerida) (Constantino et al., 1994) and *Agelas* (Agelasidae) (König & Wright, 1993). Related bromotyrosines have been found also in an ascidian, *Botryllus* (McDonald et al., 1995) and a green alga, *Avrainvillea* (Colon et al., 1987). The reported occurrence in *Hexadella* (Dendroceratida) of both bromotyrosine derivatives (Verongida) (Morris & Andersen, 1989) and topsentins (*Spongosorites* compounds) (Morris & Andersen, 1990) is one of the more intriguing inconsistencies.

Sterols, fatty acids, carotenoids, nucleosides and sesquiterpene quinones have been reported across families and genera. Their value for taxonomy is low. The apparent absence of cyclic peptides and macrolides in this order is noteworthy.

Calcarea compounds. Approximately 40 secondary metabolites were reported from at least 9 species. So far the subclass Calcaronea did not yield any compounds (the record of phospholipid fatty acids and sterols from Caribbean

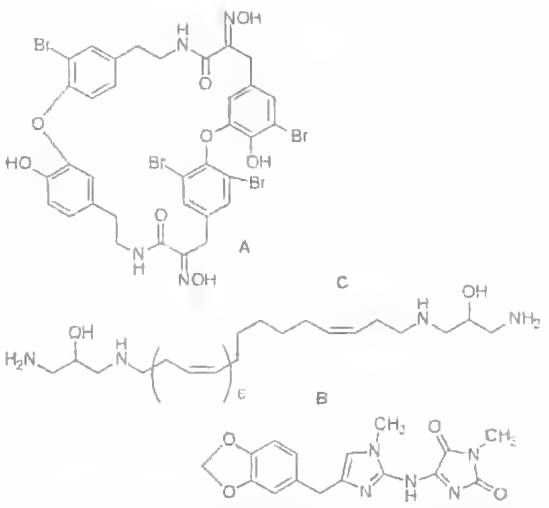

FIG. 9. A, bastadine from *Ianthella basta*. B, clathridine from *Clathrina clathrus*. C, rhapsamine from *Leucetta leptorhaphis*.

TABLE 4. Chemical markers (bold print) and non-exclusive markers (plain print) for sponge taxa, with numbers of species from which the compound was isolated. Figure numbers refer to figures presented in this review. For further comments and explanations see text.

Sponge group (No. spp. studied)	Compound type (example of structure)
Homosclerophorida (9)	**peroxy-polyketides** (Fig. 3E)
Plakina-Corticium (2)	**steroid-amines** (Fig. 3F)
Astrophorida (8)	saponines (Fig. 4A)
Stelletta s.l. (4)	**triterpenes** (Fig. 4B)
Penares (2)	**penaresidins** (Fig. 4C)
Pachastrellidae (2)	sulfated sterols (Fig. 5E)
Suberitidae (3)	**aaptamines** (Fig. 4D)
Spirastrellidae/Clionidae (2)	4,8,12-trimethyltridecanoic acid
Cliona (2)	**clionamides** (Fig. 4E)
Latrunculiidae (4)	peroxy-sesterterpenoids (Fig. 5A)
Latrunculiidae (5)	pyrroloquinoline alkaloids (Fig. 5B)
Axinellidae- Agelasidae-Ceratoporellidae (26)	**pyrrole-2-carboxylic derivatives** (Fig. 5C)
Axinellidae-Bubaridae-Halichondriidae (32)	**isocyanoterpenes** (Fig. 5D)
Halichondriidae (9)	sulfated sterols (Fig. 5E)
Desmoxyidae (3)	**cyclic diterpenes** (Fig. 5F)
Myrmekioderma (2)	linear diterpenes (Fig. 6A)
Didiscus (2)	sesquiterpene phenols (Fig. 6B)
Spongosorites (4)	topsentins (Fig. 6C)
Agelas (6)	**di- and sesquiterpenes** (Fig. 6D)
Crambeidae (3)	**polycyclic guanidine alkaloids** (Fig. 6F)
Mycale (5)	peroxy-sesterterpenoids (Fig. 7A)
Trikentrion (2)	**trikentrin indoles** (Fig. 7B)
Haplosclerida s.l. (ca. 17)	**straight-chain acetylenes** (Figs 7C-D)
Haplosclerida s.l. (ca. 22)	**3-alkylpiperidine derivatives** (Figs 7E-F)
Petrosia (ca. 7)	**polyhydroxylated acetylenes** (Fig. 7C)
Xestospongia s.s. (ca. 3)	**brominated acetylenes** (Fig. 7D)
Niphatidae + Callyspongiidae (ca. 6)	**linear 3-alkylpiperidines** (Fig. 7E)
Chalinidae + Petrosiidae (ca. 8)	**cyclic 3-alkylpiperidines** (Fig. 7F)
Petrosiidae + Phloeodictyidae (8)	cyclopropene sterols (Fig. 7G)
Haliclona (2)	tetrahydropyrans (Fig. 8A)
Dictoceratida + Dendroceratida (102)	**furano-or lactone terpenes** (Figs 8B-D)
Spongiidae + Thorectidae + Irciniidae (56)	furano-or lactone sesterterpenes (Fig. 8B)
Dysideidae (14)	furano-or lactone sesquiterpenes (Fig. 8C)
Darwinellidae + Dictyodendrillidae (13)	furano - or lactone diterpenes (Fig. 8D)
Verongida (22)	**bromotyrosine derivatives** (Fig. 8E)
Ianthella (2)	macrocyclic bromotyrosines (Fig. 9A)
Clathrinida (4)	**guanidine-imidazoles** (Fig. 9B)
Clathrinida (3)	**long-chained aminoalcohols** (Fig. 9C)

Leucosolenia canariensis (Carballeira & Shalabi, 1995) almost certainly concerns *Clathrina*, which is a member of the Calcinea). Within the subclass Calcinea compounds were isolated only from members of the order Clathrinida.

Guanidine-imidazoles (e.g. Ciminiello et al., 1989) (Fig. 9B) are recorded across families: from 1 species of *Clathrina* (Clathrinidae) and 3 species of *Leucetta* (Leucettidae), and are thus markers for the order Clathrinida.

Long-chained aminoalcohols (Jayatilake et al., 1997) (Fig. 9C) are recorded across families: from 1 species of *Clathrina* (Clathrinidae) and 2 species of *Leucetta* (Leucettidae), and are thus also markers for the order Clathrinida.

Sterols, fatty acids, a macrolide (similar to those of various Demospongiae) and a pteridine (similar to compounds from terrestrial organisms) make up the remaining compounds reported from

Calcarea. These do not appear to have chemotaxonomic significance.

SUMMARY

Table 4 summarises the conclusions, showing that a total of 38 chemical markers have been identified for 35 sponge groups of different taxonomic levels (7 orders, 8 family groups, 7 families and 13 genera). However, only 22 of these markers show some consistency, the remaining 16 presenting substantial inconstencies preventing their use as reliable markers ('non-exclusive markers'). The overview presented above clearly demonstrates that, despite huge numbers of compounds isolated from sponges, only a fraction shows potential as chemical markers for larger or smaller groups. When the distribution of the thousands of compounds over the various sponge groups is viewed as a whole, most demonstrate an erratic, scattered distribution, occurring either in single species only, or shared by unrelated species.

Thus, the 22 markers and 16 non-exclusive markers comprise only a small part of the chemical database. Moreover, markers often are not well-founded because only a handful of species have so far been recorded to contain them. Further exploration may or may not establish their consistent occurrence in the group.

Nevertheless, about 260 sponge species (out of a total of about 475) appear to contain secondary metabolites belonging to the 22 markers with utility for sponge chemosystematics.

DISCUSSION

CHEMISTRY AS A CHARACTER FOR SYSTEMATICS. The major problem preventing the use of chemotaxonomic markers as characters for sponge classification is their lack of consistency. Not one marker is problem-free, either because it is reported to occur outside the group for which it is supposed to be a marker, or because the marked groups overlap partially. To be useful for classification, markers should either include each others groups or exclude them completely.

Seemingly solid markers known from dozens of closely related species in a particular taxonomic group have also been reported from a few sponges phylogenetically unrelated from that particular sponge group. Examples include: bromotyrosine compounds (Verongida markers) in the poecilosclerid *Iotrochota*; furanoterpenes (Dictyo- and Dendroceratida markers) in the haplosclerid *Amphimedon*; straight-chain acetylenes (Haplosclerida markers) in halichondrid *Phakellia* and poecilosclerid *Raspailia*; isocyanoterpenes (Halichondrida marker) in the haplosclerid *Amphimedon*.

The same lack of consistency is apparent when the distribution of marker derivatives is viewed within the group of which they are assumed to be characteristic. Examples include: furanosesterterpenes concentrated in Dictyoceratida overlap in distribution with furanoditerpenes concentrated in Dendroceratida (as discussed in detail above); linear 3-alkylpiperidine derivatives concentrated in Callyspongiidae and Niphatidae overlap in distribution with macrocycle 3-alkylpiperidines concentrated in Chalinidae and Petrosiidae; isocyano-diterpenes, concentrated in most Halichondrida s.l. (including Axinellidae) are lacking in Agelasidae.

These inconsistencies may be the result of one or more of the following four explanations.

Parallel biosynthetic pathways. These may lead to compounds with structural similarity. Secondary metabolites are built from very generally distributed precursors of the primary metabolism. Different enzymes may have the property for allowing the biosynthesis of structurally related compounds. Such an explanation may be valid for the occurrence of bromotyrosine derivatives in *Iotrochota*, a genus which cannot conceivably be mistaken for a verongid. Tyrosine and bromine are very general molecules in marine organisms and their combination may be achieved by different enzymes. This explanation, however, requires empirical support through biosynthetic experiments.

Microsymbiont involvement. Although evidence for the involvement of microsymbionts in production of sponge secondary metabolites is largely circumstantial, several studies have established that compounds suspected to be of microsymbiont origin were indeed produced by bacteria and fungi isolated from sponges. Examples of sponges known to harbour microsymbionts which are the source of bioactive compounds are: *Theonella swinhoei*, *Halichondria okadai*, *Mycale* sp., *Tedania ignis*, *Calyx podatypa*, *Dysidea herbacea* and *Darwinella rosacea*. Schmitz (1994) provides a review of such proven or suspected cases.

On the basis that compounds are universally inconsistently distributed, it is conceivable that all natural products isolated from sponges are of microsymbiont origin. Studies which have allegedly identified sponge cells as the source of

a given compound (Faulkner et al., 1994; Garson, 1994; Uriz et al., 1996) did not address the real possibility that isolated sponge cell fractions, free from bacterial cells, contained endosymbionts ultimately involved in the production of compounds.

Microsymbionts involved in natural products biosynthesis may be species- or group-specific, in which case a close correspondence between sponge phylogeny and compound type is most likely. Such cases would not be easily distinguished from true sponge cell origin of compounds. It is possible that symbionts may be occasionally transferred to other organisms, including other sponges, which would explain 'pockets' of concentrated occurrence of compound types in unrelated organisms.

Careless specimen handling. During earlier days of natural products exploration, in particular, collected specimens were not always treated in a way required to get unequivocal results. Specimens that were not 'cleaned' from overgrowing algae and epizootic invertebrates were quoted as sources for compounds not actually produced by them. There are quite a few of these suspected cases, which can only be solved if a voucher including the epibionts has been retained. Labels have also been confused, resulting in reciprocal mismatch of compounds and sponge identities. Such cases are unlikely to be easily solved and will continue to 'pollute' the database.

Incorrect identification/classification. Some sponge groups are extremely difficult to identify to family or genus level and considerable taxonomic experience is needed. Moreover, classification of such groups is often a source of disagreement among reigning classification systems. Specimens have often been identified by dozens of taxonomists with very diverse experiences and views on the classification, resulting in an almost Babylonic confusion of sponge sources of interesting chemistry, especially in certain genera (e.g. *Batzella*, *Hymeniacidon*, *Halichondria*, *Amphimedon*, *Reniera*, *Xestospongia* and *Cribrochalina*). It is obvious that re-examination of vouchers — if at all retained — is needed to correct the more obvious mistakes. It is essential that voucher specimens of important sources of compounds be lodged in museums or collections maintained in perpetuity, and not thrown away after a certain period.

IMPACT OF CHEMISTRY ON CURRENT CLASSIFICATION. Several taxonomists have used chemical data to underbuild existing classifications or to support proposals for changes in the classification. Bergquist (1978) erected the Verongida on the basis of the universal occurrence of bromotyrosines in the group, which was earlier recognised only at the family level. This proposal remains unchallenged. Bergquist (1980) erected Nephcliospongida (later to be renamed Petrosida for nomenclatorial reasons), based on the occurrence of cyclopropene sterols. This proposal has been challenged on morphological (e.g. Van Soest, 1990) as well as chemical grounds (Fromont et al., 1994). Recent chemosystematic analyses (Andersen et al., 1996; Van Soest et al., 1998) yielded strong chemical arguments for the integrity of the Haplosclerida s.l. Van Soest (1991) also used chemical data to unite Axinellidae and Halichondriidae into a Halichondrida s.l. Braekman et al. (1992) on the basis of chemical arguments, suggested including Agelasidae into that group, although without making a formal proposal to do so. Chemical data are additional proof that soft bodied *Agelas* and the sclerosponges *Goreauiella* and *Astrosclera* are closely related, supporting morphological indications (Rinehart, 1989; Braekman et al., 1992; Williams & Faulkner, 1996).

It appears as if the chemosystematic evidence for order-level relationships are more-or-less exhausted. A final proposal could be made to formally unite Dictyoceratida and Dendroceratida into a taxon of the ordinal level, because both groups contain furanoterpenes. Such a proposal has the added advantage of avoiding border disputes at the ordinal level over the position of *Dysidea* (Boury-Esnault et al., 1990; Bergquist, 1996). Further rearrangement of suborders, families and genera may well be necessary, but will remain within unchallenged ordinal boundaries.

Promising chemosystematic conclusions may be expected in the near future especially at the genus level. Examples are *Crambe* - *Monanchora* (Poecilosclerida: Crambeidae) sharing the same compounds; some morphological subgroups of *Xestospongia* (Haplosclerida: Petrosiidae) appear to share similar chemistry. Intriguing problems to be solved are *Latrunculia* (Hadromerida or Poecilosclerida) - *Mycale* (Poecilosclerida) relationships, which share similar chemistry but lack morphological correspondence.

CHEMOSYSTEMATICS AS A FUTURE DISCIPLINE. It seems imperative that microsymbiont involvement in the production of

compounds used to underpin classifications is investigated exhaustively. It goes without saying that classifications of sponges should be based on hypotheses of evolutionary developments in the group and not — unwittingly — on those of microsymbionts. Techniques to investigate whether or not microsymbionts are involved in this process are available, but these are sophisticated, and lie beyond the reach of the average sponge taxonomist. Thus, certainty of this outcome will be slow in arriving and may depend heavily on non-relevant factors, such as pharmacological interest in the compounds. In view of the observed widespread inconsisteneies it is judged to be unwise at the present time to propose new classification schemes that depend heavily on chemical information. Conversely, however, in the face of overwhelming morphological evidence, support from chemistry should also be underlined.

In cases where microsymbionts are demonstrated to be the source of the compounds, chemosystematic conclusions may still be possible on the basis that many microsymbionts may be obligatory and co-evolved with their sponge hosts. However, different analytical techniques are necessary to arrive at such conclusions, which involve 'mapping' phylogenetic data of microsymbionts on those of the sponge hosts (see for example Van Soest et al., 1998).

Chemosystematic studies are hampered by certain aspects of current practice of natural products chemical research. Examples are: bias caused by limited biosassays and extraction procedures, and a widespread reluctance to report on the re-discovery of already known structures. Future studies would benefit greatly from broad-spectrum bioassays including organisms or cell-lines from all five kingdoms, to maximise efforts of discovery of useful compounds. Natural products chemists should report their results, irrespective of the news value for chemists, through networks and databases. Confirmation of repeated occurrence of particular compounds in particular sponges has much greater strength of conviction.

Chemosystematic conclusions should take into account inconsistent results of previous studies. Rather than dismissing inconvenient data, it should be attempted to find out why inconsistencies are there on a case-by-case basis (parallel biosynthetic pathways, microsymbiont involvement, careless specimen handling, or mistaken identification). Such attempts can be made only fruitful in cooperative efforts of taxonomists, chemists, cell biologists and microbiologists.

LITERATURE CITED

ABRELL, L.M., BORGESON, B. & CREWS, P. 1996. A new polyketide, secocurvularin, from the saltwater culture of a sponge-derived fungus. Tetrahedron Letters 37: 8983-8984.

ALBRIZIO, S., FATTORUSSO, E., MAGNO, S., MANGONI, A. & PANSINI, M. 1992. Linear diterpenes from the Caribbean sponge *Myrmekioderma styx*. Journal of Natural Products 55: 1287-1293.

ANDERSEN, R.J., SOEST, R.W.M. VAN & KONG, F. 1996. 3-Alkylpiperidine alkaloids isolated from marine sponges in the order Haplosclerida. Pp. 301-356. In Pelletier, S.W. (ed.) Alkaloids: Chemical and biological perspectives. Vol. 10. (Pergamon: Oxford).

BAKER, B.J. 1996. ß-Carboline and isoquinoline alkaloids from marine organisms. Pp. 357-407. In Pelletier, S.W. (ed.) Alkaloids: Chemical and biological perspectives. Vol. 10. (Pergamon: Oxford).

BARROW, R.A., MURRAY, L.M., LIM, T.K. & CAPON, R.J. 1996. Mirabilins (A-F): new alkaloids from a southern Australian marine sponge, *Arenochalina mirabilis*. Australian Journal of Chemistry 49: 767-773.

BARTIK, K., BRAEKMAN, J.C., DALOZE, D., STOLLER, C., HUYSECOM, J., VAN DE VYVER, G. & OTTINGER, R. 1987. Topsentins, new toxic bisindole alkaloids from sponge *Topsentia genitrix*. Canadian Journal of Chemistry 65: 2118-2121.

BERGQUIST, P.R. 1978. Sponges. (Hutchinson: London).

1979. Sponge chemistry – A review. Pp. 383-392. In Lévi, C. & Boury-Esnault, N. (eds) Biologie des spongiaires. Éditions du Centre National de la Recherche Scientifique (291) (CNRS: Paris).

1980. The ordinal and subclass classification of the Demospongiae (Porifera): appraisal of the present arrangement, and proposal of a new order. New Zealand Journal of Zoology 7: 1-6.

1996. The marine fauna of New Zealand: Porifera: Demospongiae Part 5. Dendroceratida and Halisarcida. New Zealand Oceanographic Memoirs 107: 7-53.

BERGQUIST, P.R. & WELLS, R.J. 1983. Chemotaxonomy of the Porifera. The development and current status of the field. Pp. 1-50. In Scheuer, P.J. (ed.) Marine natural products. Vol. 5. (Academic Press: New York).

BERGQUIST, P.R., LAWSON, M.P., LAVIS, A. & CAMBIE, R.C. 1984. Fatty acid composition and the classification of the Porifera. Biochemical Systematics and Ecology 12: 63-84.

BERGQUIST, P.R., LAVIS, A. & CAMBIE, R.C. 1986. Sterol composition and the classification of the

Porifera. Biochemical Systematics and Ecology 14: 105-112.

BERGQUIST, P.R., CAMBIE, R.C. & KERNAN, M.R. 1991. Aaptamine, a taxonomic marker for sponges of the order Hadromerida. Biochemical Systematics and Ecology 19: 289-290.

BERLINCK, R.G.S., BRAEKMAN, J.C., DALOZE, D., HALLENGA, K., OTTINGER, R., BRUNO, I. & RICCIO, R. 1990. Two new guanidine alkaloids from the Mediterranean sponge *Crambe crambe*. Tetrahedron Letters 31: 6531-6534.

BLUNT, J.W. & MUNRO, M.H.G. 1998. MarinLit. A database of the literature on marine natural products for use on a Macintosh computer prepared and maintained by the Marine Chemistry Group (Department of Chemistry, University of Canterbury: Canterbury, New Zealand).

BOBZIN, S.C. & FAULKNER, D.J. 1989. Diterpenes from the marine sponge *Aplysilla polyrhaphis* and the dorid nudibranch *Chromodoris norrisi*. Journal of Organic Chemistry 54: 3902-3907.

BOURY-ESNAULT, N., VOS, L. DE, DONADEY, C. & VACELET, J. 1990. Ultrastructure of choanosome and sponge classification. Pp. 237-244. In Rützler, K. (ed.) New perspectives in sponge biology. (Smithsonian Institution Press: Washington DC).

BRAEKMAN, J.C., DALOZE, D., STOLLER, C. & SOEST, R.W.M. VAN 1992. Chemotaxonomy of *Agelas* (Porifera: Demospongiae). Biochemical Systematics and Ecology 20: 417-431.

BURRESON, B.J., CHRISTOFERSON, C. & SCHEUER, P.J. 1975. Co-occurrence of two terpenoid isocyanide-formamide pairs in a marine sponge (*Halichondria* sp.). Tetrahedron 31: 2015-2018.

CAPON, R.J., MACLEOD, J.K. & SCAMMELLS, P.J. 1986. The trikentrins, novel indoles from the sponge *Trikentrion flabelliforme*. Tetrahedron 42: 6545-6550.

CAPON, R.J. & MACLEOD, J.K. 1987. Structural and stereochemical studies on marine norterpene peroxides; part 2. Journal of Natural Products 50: 225-229.

CAPON, R.J., MACLEOD, J.K. & WILLIS, A.C. 1987. Trunculins A and B: norsesterterpene cyclic peroxides from a marine sponge; *Latrunculia brevis*. Journal of Organic Chemistry 52: 339-342.

CARBALLEIRA, N.M., MALDONADO, M.E., RIVERA, E. & PORRAS, B. 1989. The fatty acid 4,8,12-trimethyltrideeanoic as a common constituent of the phospholipids of the sponge families Spirastrellidae and Clionidae. Biochemical Systematics and Ecology 17: 311-314.

CARBALLEIRA, N.M. & SHALABI, F. 1995. The rare Caribbean sponge *Leucosolenia canariensis*: phospholipid fatty acids and sterols. Lipids 30: 467-470.

CARMELY, S., ROLL, M., LOYA, Y. & KASHMAN, Y. 1989. The structure of eryloside A, a new antitumour and antifungal 4-methylated steroidal glycoside from the sponge *Erylus lendenfeldi*. Journal of Natural Products 52: 167-170.

CARNEY, J.R., SCHEUER, P.J. & KELLY-BORGES, M. 1993. A new bastadin from the sponge *Psammaplysilla purpurea*. Journal of Natural Products 56: 153-157.

CATALAN, C.A.M., LAKSHMI, V., SCHMITZ, F.J. & DJERASSI, C. 1982. Minor and trace sterols from marine invertebrates, 39. Cyclocholest-5-en-3B-ol; a novel cyclopropyl sterol from *Spheciospongia*. Steroids 40: 455-463.

CIMINIELLO, P., FATTORUSSO, E., MAGNO, S. & MANGONI, A. 1989. Clathridine and its zinc complex, novel metabolites from the marine sponge *Clathrina clathrus*. Tetrahedron 45: 3873-3878.

CIMINIELLO, P., CONSTANTINO, V., FATTORUSSO, E., MAGNO, S. & MANGONI, A. 1992. Haliclonol, a new tetrahydropyranone from the Caribbean sponge *Haliclona hogarthi*. Heterocycles 34: 765-770.

CIMINIELLO, P., FATTORUSSO, E., FORINO, M., MAGNO, S. & PANSINI, M. 1997. Chemistry of Verongida sponges. 8. Bromocompounds from the Mediterranean sponges *Aplysina acrophoba* and *Aplysina cavernicola*. Tetrahedron 53: 6565-6572.

COLON, M., GUEVARA, P., GERWICK, W.H. & BALLANTINE, D. 1987. 5'-Hydroxyisoavrainvilleol, a new diphenylmethane derivative from the tropical green alga *Avrainvillea nigricans*. Journal of Natural Products 50: 368-374.

CONSTANTINO, V., FATTORUSSO, E., MANGONI, A. & PANSINI, M. 1994. Three new brominated and iodinated tyrosine derivatives from *Iotrochota birotulata*, a non-Verongida sponge. Journal of Natural Products 57: 1552-1556.

D'AURIA, M.V., PALOMA, L.G., MINALE, L., RICCIO, R., ZAMPELLA, A. & DEBITUS, C. 1993. Metabolites of the New Caledonian sponge *Cladocroce incurvata*. Journal of Natural Products 56: 418-423.

DUMDEI, E.J., BLUNT, J.W., MUNRO, H.M.G., BATTERSHILL, C.N. & PAGE, M.J. 1998. The whys and whats of sponge chemistry: why chemists extract sponges and what problems this cause. Pp. 353-364. In Watanabe, Y. & Fusetani, N (eds) Sponge sciences. Multidisciplinary perspectives. (Springer: Tokyo).

EDER, C., SCHUPP, P., PROKSCH, P., WRAY, V., STEUBE, K., MÜLLER, C.E., FROBENIUS, W., HERDERICH, M. & SOEST, R.W.M. VAN 1998. Bioactive pyridoacridine alkaloids from the Micronesian sponge *Oceanapia* sp. Journal of Natural Products 61: 301-305.

FAULKNER, D.J., UNSON, M.D. & BEWLEY, C.A. 1994. The chemistry of some sponges and their symbionts. Pure and Applied Chemistry 66(10/11): 1983-1990.

FROMONT, J., KERR, S., KERR, R., RIDDLE, M. & MURPHY, P. 1994. Chemotaxonomic relationships within, and comparisons between, the orders Haplosclerida and Petrosida (Porifera: Demospongiae) using sterol complements. Biochemical Systematics and Ecology 22: 735-752.

FUSETANI, N., MATSUNAGA, S. & KONOSU, S. 1981. Halistanol sulfate; an antimicrobial steroid sulfate from *Halichondria* cf. *moorei* Bergquist. Tetrahedron Letters 22: 1985-1988.

FUSETANI, N., KATO, Y., MATSUNAGA, S. & HASHIMOTO, K. 1983. Polyacetylene alcohol; inhibitor of cell division in fertilized sea urchin eggs; from *Petrosia* sp. Tethrahedron Letters 24: 2771-2774.

FUSETANI, N. & MATSUNAGA, S. 1993. Bioactive sponge peptides. Chemical Review 93: 1793-1806.

GARSON, M.J. 1994. The biosynthesis of sponge secondary metabolites: Why it is important. Pp. 427-440. In Soest, R.W.M. van, Kempen, T.M.G. van & Braekman, J.C. (eds) Sponges in time and space. (Balkema: Rotterdam).

GIULIO, A. DE, ROSA, S. DE, VICENZO, G. DI & ZAVODNIK, N. 1989. Terpenoids from the North Adriatic sponge *Spongia officinalis*. Journal of Natural Products 52: 1258-1262.

GREEN, G. 1977. Ecology of toxicity in marine sponges. Marine Biology 40: 207-215.

GUELLA, G., MANCINI, I., GUERRIERO, A. & PIETRA, F. 1985. New furano-sesquiterpenoids from Mediterranean sponges. Helvetica Chimica Acta 68: 1276-1282.

HERB, R., CARROLL, A.R., YOSHIDA, W.Y., SCHEUER, P.J. & PAUL, V.J. 1990. Polyalkylated cyclopentindoles: cytotoxic fish antifeedants from a sponge, *Axinella* sp. Tetrahedron 46: 3089-3092.

HIGGS, M.D. & FAULKNER, D.J. 1978. Plakortin; an antibiotic from *Plakortis halichondrioides*. Journal of Organic Chemistry 43: 3454-3457.

ISHIBASHI, M., KUROSAKI, M., MIKAMI, Y. & KOBYASHI, J. 1993. The stereochemistry of variabilin, an antimocrobial sesterterpene isolated from marine sponges. Natural Products Letters 3: 189-192.

ISHIDA, K., ISHIBASHI, M., SHIGEMORI, H., SASAKI, T. & KOBAYASHI, J. 1992. Agelasine G, a new antileukemic alkaloid from the Okinawan marine sponge *Agelas* sp. Chemical Pharmacological Bulletin. 40: 766-767.

ITOH, T., SICA, D. & DJERASSI, C. 1983. Minor and trace sterols. 36. (24S)-24H-Isocalysterol; a new steroidal cyclopropene from sponge *Calyx nicaensis*. Journal of Organic Chemistry 48: 890-892.

JAYATILAKE, G.S., BAKER, B.J. & McCLINTOCK, J.B. 1997. Rhapsamine, a cytotoxin from the Antarctic sponge *Leucetta leptorhaphis*. Tetrahedron Letters 38: 7507-7510.

JUREK, J., SCHEUER, P.J. & KELLY-BORGES, M. 1994. Two steroidal alkaloids from a sponge, *Corticium* sp. Journal of Natural Products 57: 1004-1007

KERR, R.G. & KELLY-BORGES, M. 1994. Biochemical and morphological heterogeneity in the Caribbean sponge *Xestospongia muta* (Petrosida: Petrosiidae). Pp. 65-74. In Soest, R.W.M. van, Kempen, T.M.G. van & Braekman, J.C. (eds) Sponges in time and space. (Balkema: Rotterdam).

KOBAYASHI, J., MURAYAMA, T., KOSUGE, F., ISHIBASHI, M., KOBAYASHI, H., OHIZUMI, Y., OHTA, T., NOZOE, S. & SASAKI, T. 1990. Niphatesines A-D, new antineoplastic pyridine alkaloids from the Okinawan marine sponge *Niphates* sp. Journal of the Chemical Society Perkin Transactions 1: 3301-3303.

KOBAYASHI, J., CHENG, J., ISHIBASHI, M., WALCHLI, M.R., YAMAMURA, S. & OHIZUMI, Y. 1991. Penaresidin A and B, two novel azetidine alkaloids with potent actomyosin ATPase-activating activity from the Okinawan marine sponge *Penares* sp. Journal of the Chemical Society Perkin Transactions 1: 1135-1137

KOBAYASHI, M. & KITAGAWA, I. 1998. Likely microbial participation in the production of bioactive marine sponge chemical constituents. Pp. 379-389. In Watanabe, Y. & Fusetani, N (eds) Sponge sciences. Multidisciplinary perspectives. (Springer: Tokyo).

KOMOTO, S. & MCCONNELL, O.J. 1988. New anticancer indole alkaloids from marine sponges. European Patent Applications 111: 1-10.

KONDO, K., SHIGEMORI, H., KIKUCHI, Y., ISHIBASHI, M., SASAKI, T. & KOBAYASHI, J. 1992. Ircinals A and B from the Okinawan marine sponge *Ircinia* sp.: plausible biogenetic precursors of manzamine alkaloids. Journal of Organic Chemistry 57: 2480-2483.

KONG, F. & ANDERSEN, R.J. 1996. Polymastiamides B-F, novel steroid/aminoacid conjugates isolated from the Norwegian marine sponge *Polymastia boletiformis*. Journal of Natural Products 59: 379-385.

KÖNIG, G.M. & WRIGHT, A.D. 1993. Agelorin-A and Agelorin-B, and 11-epi-fistularin-3, three new antibacterial fistularin-3 derivatives from the tropical marine sponge *Agelas oroides*. Heterocycles 36: 1351-1358.

LI, H., MATSUNAGA, S. & FUSETANI, N. 1995. Halicylindramides A-C, antifungal and cytotoxic depsipeptides from the marine sponge *Halichondria cylindrata*. Journal of Medical Chemistry 38: 338-343.

LIAAEN-JENSEN, S, RENSTROEM, B., RAMDAHL, T., HALLENSTVET, M. & BERGQUIST, P.R. 1982. Carotenoids of marine sponges - Accumulation and transformation highest in Poecilosclerida and Axinellida. Biochemical Systematics and Ecology 10: 167-174.

MANES, L.V., BAKUS, G.J. & CREWS, P. 1984. Bioactive marine sponge norditerpene and sesterpene peroxides. Tetrahedron Letters 25: 931-934.

McCABE, T., CLARDY, J., MINALE, L., PIZZA, C., ZOLLO, F. & RICCIO, R. 1982. A triterpenoid pigment with the isomalabaricane skeleton from the marine sponge *Stelletta* sp. (Choristida). Tetrahedron Letters 23: 3307-3310.

McDONALD, L.A., SWERSEY, J.C., IRELAND, C.M., CARROLL, A.R., COLL, J.C., BOWDEN, B.F., FAIRCHILD, C.R. & CORNELL, L. 1995. Botryllamides A-D, new brominated tyrosine derivatives from styelid ascidians of the genus *Botryllus*. Tetrahedron 51: 5237-5244.

MOLINSKI, T.F., FAHY, E., FAULKNER, D.J., VAN DUYNE, G.D. & CLARDY, J. 1988. Petrosamine, a pigment from marine sponge *Petrosia* sp. Journal of Organic Chemistry 53: 1340-1341.

MORRIS, S.A. & ANDERSEN, R.J. 1989. Nitrogenous metabolites from the deep water sponge *Hexadella* sp. Canadian Journal of Chemistry 67: 677-681.

1990. Brominated bis(indole) alkaloids from the marine sponge *Hexadella* sp. Tetrahedron 46: 715-720.

NAKAMURA, H., WU, H., ABE, R., KOBAYASHI, J., OHIZUMI, Y. & HIRATA, Y. 1983. Structures of physiologically active substances from the Okinawa Sea sponges (neural blocking activity). Tennen Yuki Kagobotsu Toronkai 26: 118-125.

PATIL, A.D., KOKKE, W.C., COCHRAN, S., FRANCIS, T.A., TOMSZEK, T. & WESTLEY, J.W. 1992. Brominated polyacetylenic acids from the marine sponge *Xestospongia muta*: inhitors of HIV protease. Journal of Natural Products 55: 1170-1177.

PERRY, N.B., BLUNT, J.W., MCCOMBS, J.D. & MUNRO, H.M.G. 1986. Discorhabdin C; a highly cytotoxic pigment from a sponge of the genus *Latrunculia*. Journal of Organic Chemistry 51: 5476-5478.

PERRY, N.B., BLUNT, J.W., MUNRO, H.M.G., HIGA, T. & SAKAI, R. 1988. Discorhabdin D, an antitumour alkaloid from the sponges *Latrunculia* and *Prianos* sp. Journal of Organic Chemistry 53: 4127-4128.

PORDESIMO, E.O. & SCHMITZ, F.J. 1990. New bastadins from the sponge *Ianthella basta*. Journal of Organic Chemistry 55: 4704-4709.

PRINSEP, M.R., BLUNT, J.W. & MUNRO, H.M.G. 1989. A new sterol sulfate from the marine sponge *Stylopus australis*. Journal of Natural Products 52: 657-659.

RAVI, B.N., KOKKE, W.C.M.C., DELSETH, C. & DJERASSI, C. 1978. Isolation and structure of 26;27-cycloapsterol (petrosterol), a cyclopropane-containing sterol from sponge *Halichondria* sp. Tetrahedron Letters 1978: 4379-4380.

RINEHART, K.L. 1989. Biologically active marine natural products. Pure and Applied Chemistry 61: 525-528.

ROSSER, R. M. & FAULKNER, D.J. 1984. Two steroidal alkaloids from marine sponge; *Plakina* sp. Journal of Organic Chemistry 49: 5157-5160.

RYU, G., MATSUNAGA, S., FUSETANI, N. 1994. Bioactive marine metabolites. 64. Discodermin E, a cytotoxic and antimicrobial tetradecapeptide, from the marine sponge *Discodermia kiiensis*. Tetrahedron Letters 35: 8251-8254.

SAKAI, R., HIGA, T., JEFFORD, C.W. & BERNARDINELLI, G. 1986. Manzamine A; an antitumor alkaloid from a sponge. Journal of the American Chemical Society 108: 6404-6405.

SALOMON, C.E. & FAULKNER, D.J. 1996. Sagitol, a pyridoacridine alkaloid from the sponge *Oceanapia sagittaria*. Tetrahedron Letters 37: 9147-9148.

SARMA, A.S., DAUM, T. & MÜLLER, W.E.G. 1993. Secondary metabolites from marine sponges. (Ullstein Mosby: Berlin).

SCHMITZ, F.J. 1994. Cytotoxic compounds from sponges and associated microfauna. Pp. 485-496. In Soest, R.W.M. van, Kempen, T.M.G. van & Braekman, J.C. (eds) Sponges in time and space. (Balkema: Rotterdam).

SCHMITZ, F.J., AGARWAL, S.K., GUNASEKERA, S.P., SCHMIDT, P.G. & SHOOLERY, J.N. 1983. Amphimedine; new aromatic alkaloid from a Pacific sponge, *Amphimedon* sp. Journal of the American Chemical Society 105: 4835-4836.

SENNETT, S.H., POMPONI, S.A. & WRIGHT, A.E. 1992. Diterpene metabolites from two chemotypes of the marine sponge *Myrmekioderma styx*. Journal of Natural Products 55: 1421-1429.

SILVA, C.J. & DJERASSI, C. 1991. Sterols in marine invertebrates. Part 64. Isolation, stereochemistry and biosynthesis of sormosterol, a novel cyclopropane-containing sponge sterol. Collected Czech Chemical Communications 56: 1093-1105.

SOEST, R.W.M. VAN 1980. Marine sponges from Curaçao and other Caribbean localities. Part II. Haplosclerida. Studies on the Fauna of Curaçao and other Caribbean Islands 62: 1-173.

1990a. Toward a phylogenetic classification of sponges. Pp. 344-348. In Rützler, K. (ed.) New perspectives in sponge biology. (Smithsonian Institution Press: Washington).

1990b. Demosponge higher taxa classification re-examined. Pp. 54-71. In Reitner, J. & Keupp, H. (eds) Fossil and recent sponges. (Springer Verlag: Heidelberg).

1994. Demosponge distribution patterns. Pp. 213-224. In Soest, R.W.M. van, Kempen, T.M.G. van & Braekman, J.C. (eds) Sponges in time and space. (Balkema: Rotterdam).

SOEST, R.W.M. VAN, DIAZ, M.C. & POMPONI, S.A. 1990. Phylogenetic classification of the

Halichondrids (Porifera, Demospongiae). Beaufortia 40: 15-62.

SOEST, R.W.M. VAN, BRAEKMAN, J.C., FAULKNER, D.J., HAJDU, E., HARPER, M.K. & VACELET, J. 1996. The genus *Batzella*: a chemosystematic problem. Bulletin de l'Institut Royal des Sciences Naturelle de Belgique 66(suppl.): 89-101.

SOEST, R.W.M. VAN, FUSETANI, N. & ANDERSEN, R.J. 1998. Straight-chain acetylenes as chemostaxonomic markers of the marine Haplosclerida. Pp. 3-30. In Watanabe, Y. & Fusetani, N. (eds) Sponge sciences. Multidisciplinary perspectives. (Springer: Tokyo).

STIERLE, D.B. & FAULKNER, D.J. 1979. Metabolites of the marine sponge *Chondrosia collectrix*. Journal of Organic Chemistry 44: 964-968.

STIERLE, A.A., CARDELLINA, J.H. & SINGLETON, F.L. 1991. Benzothiazoles from a putative bacterial symbiont of the marine sponge *Tedania ignis*. Tetrahedron Letters 32: 4847-4848.

STONARD, R.J. & ANDERSEN, R.J. 1980. Linear peptide alkaloids from the sponge *Cliona celata* (Grant). Celenamides C and D. Canadian Journal of Chemistry 58: 2121-2126.

SUPRIYONO, A., SCHWARZ, B., WRAY, V., WITTE, L., MÜLLER, W.E.G., SOEST, R.W.M. VAN, SUMARYONO, W. & PROKSCH, P. 1995. Bioactive alkaloids from the tropical marine sponge *Axinella carteri*. Zeitschrift für Naturforschung 50c: 669-674.

TOTH, S.I. & SCHMITZ, F.J. 1994. Two new cytotoxic peroxide-containing acids from a New Guinea sponge *Callyspongia* sp. Journal of Natural Products 57: 123-127.

TSUKAMOTO, S., KATO, H., HIROTA, H. & FUSETANI, N. 1996. Pseudoceratine: a new antifouling spermidine derivative from the marine sponge *Pseudoceratina purpurea*. Tetrahedron Letters 37: 1439-1440.

URIZ, M.J., BECERRO, M.A., TUT, J.M. & TURON, X. 1996. Location of toxicity within the Mediterranean sponge *Crambe crambe* (Demospongiae: Poecilosclerida). Marine Biology 124: 583-590.

WELLS, R.J. 1976. A novel peroxyketal from a sponge: genus *Chondrilla*. Tetrahedron Letters 1976: 2637-2638.

WILLIAMS, D.H. & FAULKNER, D.J. 1996. N-methylated ageliferins from the sponge *Astrosclera willeyana* from Pohnpei. Tetrahedron 52 (15): 5381-5390.

WRIGHT, A.E., POMPONI, S.A., McCONNELL, O.J., KOHMOTO, S. & McCARTHY, P.J. 1987. Curcuphenol & curcudiol, sesquiterpene phenols from *Didiscus flavus*. Journal of Natural Products 50: 976-978.

WU, H., NAKAMURA, H., KOBAYASHI, J., OHIZUMI, Y. & HIRATA, Y. 1984. Agelasine -e & -f, novel monocyclic diterpenoids with 9-methyladeninium unit possessing inhibitory effects on Na,K-ATPase from Okinawan sponge *Agelas nakamurai*. Tetrahedron Letters 25: 3719-3722.